Human TNF alpha ELISA Kit

User Manual

Catalog # MBS824724

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Sandwich Enzyme-Linked Immunosorbent Assay for Quantitative Detection of Human TNF alpha Concentrations in Cell Culture Supernatants, Serum, Plasma, Urine, Tissue Homogenates.

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I. INTRODUCTION

Tumor necrosis factor-alpha (TNF-alpha, or TNF) is secreted by macrophages in response to inflammation, infection and cancer. Human Tumor Necrosis Factor (TNF) and Lymphotoxin (TNF-beta) are cytotoxic proteins which have similar biological activities and share 30% amino acid homology. TNF-alpha is produced by monocytes, which can stimulate endothelial cells to produce the multilineage growth factor granulocyte-macrophage colony-stimulating factor and extend the role of this immunoregulatory protein to the regulation of hematopoiesis in vitro. TNF is a soluble protein that causes damage to tumor cells but has no effect on normal cells. Human TNF has been purified to apparent homogeneity as a 17.3-kilodalton protein from HL-60 leukemia cells and has showed cytotoxic and cytostatic activities against various human tumor cell lines. The human TNF cDNA is 1585 base pairs in length and encodes a protein of 233 amino acids. The mature protein begins at residue 77, leaving a long leader sequence of 76 amino acids. TNF-alpha has been mapped to human chromosome 6.2

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II. ASSAY PRINCIPLES

The Human TNF alpha ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of Human TNF alpha in Cell Culture Supernatants, Serum, Plasma, Urine, Tissue Homogenates. This assay employs an antibody specific for Human TNF alpha coated on a 96-well plate. Standards and samples are pipetted into the wells and TNF alpha present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-Human TNF alpha antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of TNF alpha bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

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III. KIT COMPONENTS

Component	Volume
96-well Plate Coated With Anti-Human TNF alpha Antibody	12 x 8 Strips
Human TNF alpha Standard	1 ng x 2
Biotin-Labeled Detection Antibody (100X)	120 µl
Streptavidin-HRP (100X)	120 µl
Standard/Sample Diluent	30 ml
Detection Antibody Diluent	12 ml
Streptavidin-HRP Diluent	12 ml
Wash Buffer (20X)	30 ml
TMB Substrate Solution	12 ml
Stop Solution	12 ml
Plate Adhesive Strips	3 Strips
Technical Manual	1 Manual

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IV. STORAGE AND STABILITY

All kit components are stable at 2 to 8 °C. Standard (recombinant protein) should be stored at -20 °C or -80 °C (recommended at -80 °C) after reconstitution. Opened Microplate Wells or reagents may be store for up to 1 month at 2 to 8 °C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.
Note: the kit can be used within one year if the whole kit is stored at -20 °C. Avoid repeated freeze-thaw cycles.

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V. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Adjustable pipettes and pipette tips to deliver 2 μl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

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VI. HEALTH AND SAFETY PRECAUTIONS

1. Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment.
2. Stop Solution contains 2 N Sulfuric Acid (H_2SO_4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.

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VII. REAGENT PREPARATION

1. Sample Preparation

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

Cell culture supernates: Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

Serum: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 minutes. Analyze the serum immediately or aliquot and store samples at -20°C.

Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1500 X g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C.

Cell Lysates: Collect cells and rinse cells with PBS. Homogenize and lyse cells throughly in lysate solution. Centrifuge cell lysates at approximately 10000 X g for 5 minutes to remove debris. Aliquots of the cell lysates were removed and assayed.

Bone Tissue: Extract demineralized bone samples in 4 M Guanidine-HCl and protease inhibitors. Dissolve the final sample in 2 M Guanidine-HCl.

Tissue Homogenates: Rinse tissue with PBS to remove excess blood, chopped into 1-2 mm pieces, and homogenize with a tissue homogenizer in PBS or in lysate solution, lysate solution: tissue net weight = 10ml : 1g (i.e. Add 10ml lysate solution to 1g tissue). Centrifuge at approximately 5000 X g for 5 minutes. Assay immediately or aliquot and store homogenates at -20°C. Avoid repeated freeze-thaw cycles.

Urine: Urinary samples should be cleared by centrifugation and then can be used directly without dilution. Storage at -20°C.

2. Human TNF alpha Standard Preparation

Reconstitute the lyophilized Human TNF alpha Standard by adding 1 ml of Standard/Sample Diluent to make the 1000 pg/ml standard stock solution. Allow

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solution to sit at room temperature for 5 minutes, then gently vortex to mix completely. Use within one hour of reconstituting. Two tubes of the standard (1 ng per tube) are included in each kit. Use one tube for each experiment.

Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay (15.625 pg/ml - 1000 pg/ml) as below.

Standard/Sample Dilution Buffer serves as the zero standard (0 pg/ml).

Standard	Add	Into
1000 pg/ml		
500 pg/ml	500 µl of the Standard (1000 pg/ml)	500 µl of the Standard/Sample Diluent
250 pg/ml	500 µl of the Standard (500 pg/ml)	500 µl of the Standard/Sample Diluent
125 pg/ml	500 µl of the Standard (250 pg/ml)	500 µl of the Standard/Sample Diluent
62.5 pg/ml	500 µl of the Standard (125 pg/ml)	500 µl of the Standard/Sample Diluent
31.25 pg/ml	500 µl of the Standard (62.5 pg/ml)	500 µl of the Standard/Sample Diluent
15.625 pg/ml	500 µl of the Standard (31.25 pg/ml)	500 µl of the Standard/Sample Diluent
0 ng/ml	1 ml of the Standard/Sample Diluent	

Note: The standard solutions are best used within 2 hours. The 1000 pg/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

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3. Biotin-Labeled Detection Antibody Working Solution Preparation

The Biotin-Labeled Detection Antibody should be diluted in 1:100 with the Detection Antibody Diluent and mixed thoroughly. The solution should be prepared no more than 2 hours prior to the experiment.

4. Streptavidin-HRP Working Solution Preparation

The Streptavidin-HRP should be diluted in 1:100 with the Streptavidin-HRP Diluent and mixed thoroughly. The solution should be prepared no more than 1 hour prior to the experiment.

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5. Wash Buffer Working Solution Preparation

Pour entire contents (30 ml) of the Wash Buffer Concentrate into a clean 1,000 ml graduated cylinder. Bring final volume to 600 ml with glass-distilled or deionized water (1:20).

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VIII. ASSAY PROCEDURE

The Streptavidin-HRP Working Solution and TMB Substrate Solution must be kept warm at 37°C for 30 minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of protein amount in samples.

1. Add 100 µl of each standard and sample into appropriate wells.
2. Cover well and incubate for 90 minutes at room temperature or over night at 4°C with gentle shaking.
3. Remove the cover, discard the solution and wash plate 3 times with Wash Buffer Working Solution, and each time let Wash Buffer Working Solution stay in the wells for 1 - 2 minutes. Blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 100 µl of Biotin-Labeled Detection Antibody Working Solution into each well and incubate the plate at 37°C for 60 minutes.
5. Wash plate 3 times with Wash Buffer Working Solution, and each time let Wash Buffer Working Solution stay in the wells for 1 - 2 minutes. Discard the Wash Buffer Working Solution and blot the plate onto paper towels or other absorbent material.
6. Add 100 µl of Streptavidin-HRP Working Solution into each well and incubate the plate at 37°C for 45 minutes.
7. Wash plate 5 times with Wash Buffer Working Solution, and each time let wash buffer stay in the wells for 1 - 2 minutes. Discard the wash buffer and blot the plate onto paper towels or other absorbent material.
8. Add 100 µl of TMB Substrate Solution into each well and incubate plate at 37°C in dark for 30 minutes.
9. Add 100 µl of Stop Solution into each well. The color changes into yellow immediately.

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10. Read the O.D. absorbance at 450nm in a microplate reader within 30 minutes after adding the Stop Solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) - (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

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IX. ASSAY PROCEDURE SUMMARY

• Prepare all reagents, samples and standards
• Add 100 µl Standard or Sample
• Wash plate 3 times with Wash Buffer Working Solution
• Add 100 µl Biotin-Labeled Detection Antibody Working Solution
• Wash plate 3 times with Wash Buffer Working Solution
• Add 100 µl Streptavidin-HRP Working Solution
• Wash plate 5 times with Wash Buffer Working Solution
• Add 100 µl TMB Substrate Solution
• Add 100 µl Stop Solution
• Read the plate at 450nm

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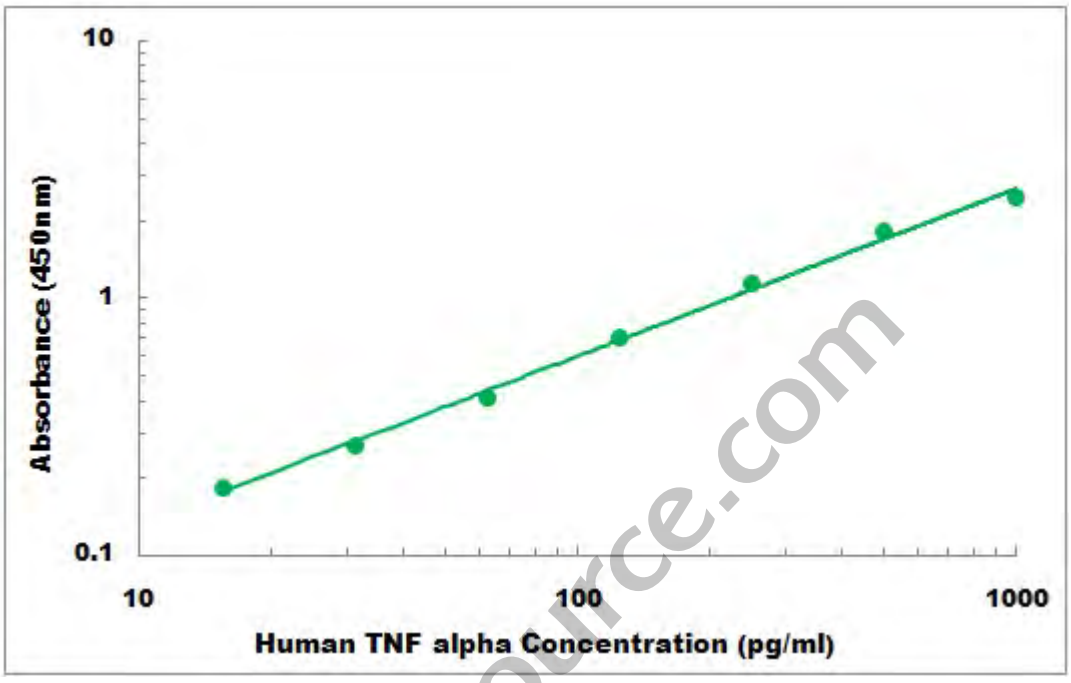
X. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



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XI. SENSITIVITY

The minimum detectable dose of Human TNF alpha is typically less than 4 pg/ml.

XII. SPECIFICITY

The Human TNF alpha ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant Human TNF alpha proteins within the range of 15.625 pg/ml - 1000 pg/ml.

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XIII. CROSS REACTIVITY

No detectable cross-reactivity with other relevant proteins.

XIV. REFERENCES

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3. Broudy, V. C.; Kaushansky, K.; Segal, G. M.; Harlan, J. M.; Adamson, J. W. Tumor necrosis factor type alpha stimulates human endothelial cells to produce granulocyte/macrophage colony-stimulating factor. Proc. Nat. Acad. Sci. 83: 7467-7471, 1986.

4. Wang, A. M.; Creasey, A. A.; Ladner, M. B.; Lin, L. S.; Strickler, J.; Van Arsdell, J. N.; Yamamoto, R.; Mark, D. F. Molecular cloning of the complementary DNA for human tumor necrosis factor. Science 228: 149-154, 1985.

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XV. TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
High signal and background in all wells	• Insufficient washing	• Increase number of washes • Increase time of soaking between in wash
	• Too much Streptavidin-HRP	• Check dilution, titration
	• Incubation time too long	• Reduce incubation time
	• Development time too long	• Decrease the incubation time before the stop solution is added
No signal	• Reagent added in incorrect order, or incorrectly prepared	• Review protocol
	• Standard has gone bad (If there is a signal in the sample wells)	• Check the condition of stored standard
	• Assay was conducted from an incorrect starting point	• Reagents allows to come to 20 - 30 °C before performing assay
Too much signal-whole plate turned uniformly blue	• Insufficient washing-unbound Streptavidin-HRP remaining	• Increase number of washes Carefully
	• Too much Streptavidin-HRP	• Check dilution
	• Plate sealer or reservoir reused, resulting in presence of residual Streptavidin-HRP	• Use fresh plate sealer and reagent reservoir for each step
Standard curve achieved but poor discrimination between point	• Plate not developed long enough	• Increase substrate solution incubation time
	• Improper calculation of standard curve dilution	• Check dilution, make new standard curve
No signal when a signal is expected, but standard curve looks fine	• Sample matrix is masking detection	• More diluted sample Recommended
Samples are reading too high, but standard curve is fine	• Samples contain protein levels above assay range	• Dilute samples and run Again
Edge effect	• Uneven temperature around work surface	• Avoid incubating plate in areas where environmental conditions vary • Use plate sealer

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XVI. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online

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